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Development of immune response during typhoid fever in man

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SUMMARY

The development of both humoral and cell-mediated immune responses (CMIR) to antigens prepared from *Salmonella typhi* was investigated in patients suffering from typhoid fever. The antibodies were determined by the standard Widal test while the leucocyte migration test was used for CMIR. These immunological parameters were correlated with the duration of illness, the duration of chloramphenicol therapy and the severity of the illness. It was found that CMIR appeared after the first week of illness in uncomplicated cases of typhoid fever, where as it remained negative in the patients who had complications. The antibody titres were similar in the two groups. On further follow up of complicated cases, the clinical recovery coincided with the development of CMIR. It may be concluded that for recovery in typhoid fever CMIR is more important than antibodies.

INTRODUCTION

Of the various mechanisms by which a vertebrate host defends itself against invasion by microbes, acquired immune responses to the antigens of infectious agents constitute an important component. In several infections humoral antibodies play a key role in protection. Development of specific antibodies during typhoid fever in man is well known (Olitzki, 1972). However, it has been documented that the free antibodies are not relevant for protection (Watson, 1957; Benneson, 1964; Hornick *et al.*, 1970). In contrast, only a little information is available on the development of specific cell-mediated immune response (CMIR) and its role in protection in this disease. In a recently reported work from this laboratory it was shown that the specific CMIR developed in majority of the patients with typhoid fever (Kumar *et al.*, 1974). However, its protective role could not be established.

The present work, a continuation of the previous study, describes the development of specific humoral antibody response as well as CMIR in patients with typhoid fever at various stages of their illness. These immune responses are correlated with the clinical picture and chloramphenicol therapy. The results indicate a possible role of specific CMIR in protection against typhoid fever.

MATERIALS AND METHODS

The study included adult and paediatric patients with blood culture positive for *Salmonella typhi.* Controls included normal healthy volunteers from amongst the staff and students of this hospital, as well as hospitalized patients who had febrile illnesses other than typhoid. For the paediatric group, the controls were taken from age- and sex-matched children with non-typhoidal febrile illnesses and from children (referred as normal) who were admitted into the surgical wards for correction of various congenital abnormalities. An extra 5 ml of blood was withdrawn during venepuncture for standard investigations. A careful history was taken in all the control subjects to exclude those who had typhoid fever or TAB vaccination over the preceding 5 years.

The development of humoral as well as cell-mediated immune response to antigens of *S. typhi* were studied in these subjects.

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Antibody titration. The antibodies to O and H antigens of *S. typhi* were determined by the standard Widal agglutination test on doubling dilutions of serum from 1:20–1:640. The readings were taken after overnight incubation at 37°C. For each group the mean titre was calculated in natural logarithms, allowing a value of 1/10 for the sera negative at 1/20. For some comparisons, groups of patients were divided into those who had 'low' or 'high' antibody titre. For this purpose, titres up to 80 were regarded as 'low' and more than 80 as 'high' for both H and O antibodies.

Leucocyte migration test. The test was done using an ultrasonic lysate of *S. typhi* as antigen prepared by the method described earlier (Kumar *et al.*, 1974). Standardization for the optimum dose of antigen used in the test was carried out on every fresh batch prepared using leucocytes from a known positive and a negative subject in the leucocyte migration test and the trypan blue dye exclusion test for determining the toxic dose. For the test, the technique of Federlin *et al.* (1971) was used with minor modifications. In short it consisted of loosely packing the capillary tubes (Gelman–Hawksley, U.K.) with leucocytes separated from defibrinated blood utilizing 2% gelatin in phosphate-buffered saline 0·5 M pH 7·4 (PBS). The cells were allowed to migrate from capillaries in perspex chambers for 16–20 hr in minimum essential medium (MEM) containing 10% foetal calf serum (Difco, U.S.A.) with or without antigen. The area of migration of cells was determined by projection and planimetry and migration index calculated by dividing the migration area in the presence of antigen with the migration area in the absence of antigen. The inherent variability of the test had been calculated for this laboratory earlier and migration index was considered positive if under 0·80.

Correlations. The results of the O and H agglutinin titres and the LMT in patients of typhoid fever were correlated with the duration of illness as well as with the duration of chloramphenicol therapy at the time of study. In addition, these immunological findings were also correlated with the severity of the clinical disease including complications.

In seven adult patients of typhoid fever it was possible to repeat the tests later and correlate them with their clinical course.

RESULTS

The study included sixty adults and twenty children with typhoid fever. For control among the adults, there were forty-one normals and twenty-nine patients with non-typhoidal febrille illnesses. The controls in paediatric age group included eleven with non-typhoidal febrile illnesses and eight who were admitted to the surgical wards for correction of some congenital abnormality. The last group was taken as normal control for paediatric age group.

The majority of the adults and all the paediatric age group typhoid cases were suffering from only a febrile illness with other usual clinical features of typhoid fever. However, in nineteen of the adult patients the disease was severe presenting with one or more complications of typhoid fever i.e. gastro-intestinal bleeding with or without perforation, jaundice, encephalopathy and peripheral vascular failure.

O and H agglutinin titres

The titres of both of these antibodies were found to be significantly higher in patients with typhoid fever (Table 1) in comparison to controls.

There was a steady rise of O and H antibody levels related to the duration of illness (Table 2). The mean titre of both O and H agglutinins in patients with typhoid of 3 weeks or more of illness was significantly elevated in comparison to the patients seen within 1st week of illness ($P<0·05$).

Chloramphenicol therapy did not prevent the development of either O or H antibodies (Table 3).

TABLE 1. O and H agglutinin titres in patients of typhoid fever and controls

Subjects	Mean O titre ± s.d.	Mean H titre ± s.d.
Typhoid (80)†	4·16 ± 1·83*	5·35 ± 1·57*
Healthy controls (49)	1·16 ± 1·06	2·32 ± 1·27
Fever controls (40)	2·15 ± 1·21	2·00 ± 1·15

* $P<0·001$ in comparison with both control.

† Figures in parentheses indicate number of cases investigated.

TABLE 2. Antibodies and LMT at different stages of typhoid fever

	Duration of illness		
	1st week	2nd week	3rd week or more
Mean O titre ± s.d.	2·91 ± 1·87	4·26 ± 1·63*	4·27 ± 2·15*
Mean H titre ± s.d.	3·27 ± 2·33	4·74 ± 1·96	5·50 ± 1·53*
Mean LMT index ± s.d.	0·91 ± 0·22	0·64 ± 0·18	0·61 ± 0·21
Proportion of patients with positive LMT	4/17 (23·5%)	24/31 (77·4%)†	26/32 (81·2%)†

* $P < 0·05$ in comparison to 1st week of illness.
† $P < 0·001$ in comparison to 1st week of illness.

TABLE 3. Correlation of the duration of chloramphenicol therapy in patients of typhoid fever with LMT and antibody titres

	Duration of therapy		
	Nil	Under 1 week	Over 1 week
Mean O titre ± s.d.	3·40 ± 1·14	4·36 ± 1·74	4·18 ± 2·13
Mean H titre ± s.d.	3·20 ± 1·64	5·64 ± 1·15*	5·82 ± 1·19*
Mean LMT index ± s.d.	0·81 ± 0·20	0·62 ± 0·24	0·54 ± 0·22*

* $P < 0·05$ in comparison to those with no chloramphenicol.

The titre of O antibodies in patients with no chloramphenicol, up to 1 week of chloramphenicol and more than 1 week of the drug did not significantly differ ($P > 0·005$). In the case of H antibodies, the titres were significantly higher ($P < 0·001$) in the two groups of patients who had been on chloramphenicol therapy. There was no association between complications of typhoid and O and H agglutinin titres (Table 4).

Leucocyte migration test

The results indicated that among the adults, forty typhoid patients out of 60 (66·6%) showed a positive LMT. Among normal adults there were sixteen out of forty-one (39·0%) and in fever controls nine out of twenty-nine (31·0%) who had a positive LMT. In children, there were fourteen out of twenty (70·0%) in the typhoid group, four out of eleven (36·3%) in fever control group and one out of eight (12·5%) in the normal control group who had a positive LMT (Fig. 1). The proportion of patients with

TABLE 4. Correlation of the severity of typhoid fever with LMT and antibody titres

		Complicated case*	Uncomplicated case	Chi. eq. P value
LMT	Positive	6	48	12·59, $P < 0·001$
	Negative	13	13	
O antibody	High	15	35	2·03, $P < 0·05$
	Low	4	26	
H antibody	High	15	46	0·00006, $P < 0·10$
	Low	4	15	

* Figures represent the number of individuals.

a positive LMT was 23·5% in the first week, 77·4% in the second week and 81·2% in third week or more of illness (Table 2). The rise in LMT positive subjects during the 2nd week or more of illness in comparison to first week of illness was highly significant ($P<0·001$). Chloramphenicol therapy did not have any effect on LMT (Table 3). The presence of complications of typhoid was inversely associated with the positive LMT and complications were more often seen in patients who did not show a positive LMT (Table 4).

It needs to be pointed out that amongst the twenty-six LMT negative typhoid fever cases, thirteen were in the first week of illness and the remaining thirteen showed one or the other complications of typhoid. There was no uncomplicated case of typhoid with a negative LMT during second week or more of illness. However, of the nineteen complicated cases, in as many as thirteen the test was negative.

After one week it was possible to repeat the LMT in seven patients whose initial test had been negative. Two of them were in the first week of illness and the remaining five were cases with complications. None of them was on corticosteroids. On retesting, four became LMT positive (including two who were in the 1st week of illness in initial testing) and all of them showed good clinical recovery. Of the remaining three which remained LMT negative, one had marked encephalopathy and was on corticosteroids for one week, second developed typhoid relapse after initial recovery and the third deteriorated with intestinal perforation and was taken away by his relatives against medical advice.

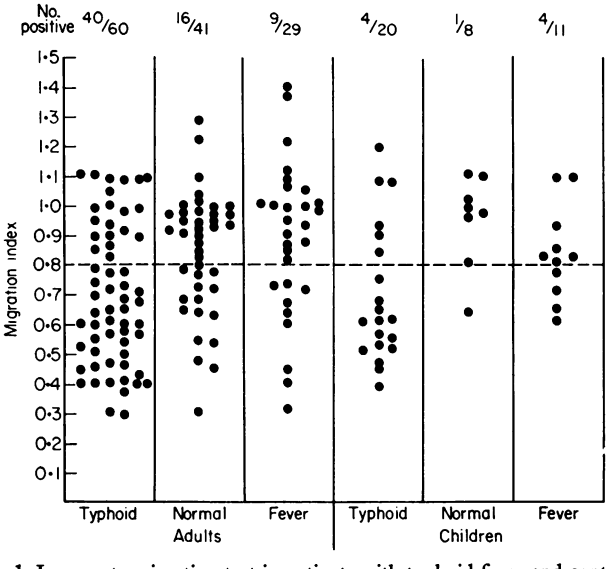

FIG. 1. Leucocyte migration test in patients with typhoid fever and controls.

DISCUSSION

The expression of protective immunity in any particular disease may depend critically on only one or a few of the wide range of effector mechanisms induced by the adaptive immune response (Nelson, 1974). As the knowledge regarding this critical protective component of the immune response in human typhoid is meagre, the present work was undertaken with the aim of investigating the mechanism of protective immunity in this disease in man. Since there is no fully effective immunizing agent available for human typhoid (WHO, 1973) it was deemed a necessary first step towards improvement of the existing typhoid vaccine.

The present work demonstrated that the development of antibodies in patients with typhoid followed the same pattern as has been extensively reported earlier and reviewed recently by Olitzki (1972). The antibodies appeared after the 1st week of illness and the titres gradually increased during the following days.

As reported by Robertson, Fathy & Wahab (1970), the present work also showed that chloramphenicol therapy did not interfere with antibody production. The higher titre of H antibody in patients who were taking chloramphenicol for more than 1 week was most likely due to the longer duration of illness. Antibody titres did not correlate with the severity of typhoid fever. It may mean that antibodies alone are not involved in recovery from this disease. Their role in association with some other factors cannot, however, be ruled out.

The leucocyte migration test, like antibodies, also became positive after the first week of illness in the patients of typhoid fever. However, once established, there was no further apparent increase in the degree of positivity during the following days. The proportion of typhoid patients giving a positive reaction in LMT during the 1st week of illness was comparable with the control groups. This background positivity was somewhat less in the paediatric age group indicating that the exposure to *S. typhi* may be a cause for this. It however needs confirmation by studying a larger number of subjects in each age group. The chloramphenicol therapy had no influence on the LMT. The comparison of LMT positive or negative state with severity of the disease gave a statistically highly significant negative correlation. The more severe cases of typhoid fever were more often LMT negative. In the uncomplicated cases of typhoid fever, LMT negative patients were all in the first week of illness. Further, during follow up the severe cases of typhoid fever with negative LMT either became LMT positive and showed clinical improvement or remained LMT negative and their clinical condition deteriorated. Thus, LMT positivity indicated good prognosis.

There is therefore a close correlation between the development of cell-mediated immunity, as estimated by the LMT test and recovery and an inverse correlation with complications and a prolonged illness in typhoid fever. These findings suggest that cell-mediated immunity may be more important than antibodies in recovery from typhoid fever.

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